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PERIODICITY IN SPIROGYRA

W. F. COPELAND

The object in beginning these studies with Spirogyra was to get notes on the rearing of species and to see how far they could be changed by conditions. With this end in view 100 aquaria were started and soon contained puzzling results. To interpret these and to get on with the experiments, it soon seemed desirable, if not necessary, to know how Spirogyra lives under natural environment. For this reason the major part of my time was given to a consideration of this plant out of doors. An attempt has been made to repeat all observations and experiments under as great a variety of conditions as possible in an ordinary biological laboratory. When field-work was not practicable or necessary, laboratory work was resumed. In this way scores of experiments have been in progress from the beginning.

After this work had continued for some months, considerations were given to conditions appearing with some regularity whether Spirogyra was being studied under natural or under artificial environment. As a possible constant condition, it was finally decided to give special attention to the subject of periodicity. In this paper only those experiments will be mentioned which seem to have some bearing on this subject. Although laboratory studies are of minor importance in the subject of periodicity, in the present discussion they will appear first.

I take this opportunity to express my obligation to those who have in any way aided me in the present study. It is a pleasure to thank Dr. C. F. HODGE, who suggested the problem, for his help and criticism; HELEN REINHERR COPELAND for help in identification of species and preparation of material for microphotographs; O. W. MILLS, Millbury, Mass., for collecting much valuable material in that locality; and Dr. O. P. DELLINGER, Worcester, Mass., for helpful suggestions and criticisms. The following persons have sent me conjugating specimens from more distant localities: W. D. HOYT and I. F. LEWIS of Johns Hopkins University; B. A. PLACE, Iowa

Wesleyan University; S. G. WINTER, Illinois Wesleyan University; and W. A. MATHENY, Sardis, Ohio.

Review of literature

In 1803 VAUCHER (6) tells of his attempt to work out the life-history of the Conjugatae. For this purpose the genus Spirogyra was used and especially the species which MÜLLER had previously described as *Conferva jugalis*. VAUCHER'S observations were made in the spring and it was found that this species would fruit and disappear as early as February 15 and as late as March 20. The zygospores which he had collected germinated July 15, and all seemed to germinate on the same day.

A history of the British freshwater algae by HASSALL (3) appeared in 1857. This well-known work contains descriptions of 42 species of Spirogyra under the name Zygnema. A single hint is given as to the time of year when conjugation takes place; namely, "The species of this group of confervae may be found in a state of conjugation during the entire spring, summer, and autumnal months, but more abundant in the spring."

In 1874 *The freshwater algae of the United States* by WOOD (9) was published in the Smithsonian Report. Speaking of the Zygnemaceae he says: "The family under consideration is among the commonest and most widely diffused of all the freshwater algae. They may be found in greater or less abundance at all seasons, but the spores appear to be formed only in the spring and early summer, at least these are the only times at which I have found fertile filaments. In the neighborhood of Philadelphia I have collected them in excellent condition as early as the beginning of April and as late as the latter part of June. Further south conjugation of course commences earlier, and fine specimens received by myself from Mr. CANBY were collected by him in Florida in February."

The work of PAUL PETIT (5) entitled *Spirogyra des environs de Paris* appeared in 1880. No general statement is made regarding the fruiting season of this genus, but, with one exception, dates are given with the description of the different species. These dates show that 20 of the 36 species described matured during March, April, and May; and all except two had matured by July. These were found in fruit from July until October. One species fruited in April and May and again in October.

Since 1880 a number of papers relating to Spirogyra have appeared, but only a few of them give any facts that can be used in a study of periodicity. BENNETT and MURRAY (1) write: "Germination sometimes takes place while still in the mother cell; but most commonly both filaments perish after conjugation, with the exception of numerous zygospores which fall to the bottom. These remain dormant through the winter . . . . germinating in the spring . . . . instances are recorded of filaments persisting through the winter. HOFMEISTER states that the growth of Spirogyra is intermittent."

The following case is reported by W. and G. S. WEST (7): "We have melted out of the ice from Micham Common, Surrey, excellent examples of *Spirogyra catenaeformis* in conjugation, the vitality of which was in nowise impaired."

The few scattered remarks given include all the notes the writer has been able to gather from papers published before 1905. In this year a few papers appeared which had a more direct bearing on the subject at hand. These seem to be the pioneer publications on the subject of algal periodicity. In indicating some of the "problems of aquatic biology" FRITSCH (2) calls attention to the fact that different bodies of water, often far apart, will have the same dominant and often the same subordinate vegetation; also that a plant may be very abundant one month and almost, if not entirely, absent the next month. An intimate relation was found to exist between the relative abundance of an alga and its period of reproduction; the formation of the sex organs frequently preceding the disappearance or diminished abundance of a given species. FRITSCH writes: "In some cases no doubt these features (maximum abundance and period of reproduction) are influenced by the periodically recurring factors such as the rise of temperature and the increase in the intensity of light in the spring; so that the maximum abundance and period of reproduction are definite phenomena."

Another paper on the subject of algal periodicity appeared in 1905. So far the publications mentioned have had to do only with freshwater algae; for that reason it is well to call attention to studies made on marine forms. While at work on Dictyotaceae, WILLIAMS

(**8**) discovered that Dictyota had definite periods for the development of its fruit. This is well explained in his introductory paragraph: "While studying the development of the sexual cells in Dictyota, the interesting discovery was made that the process was practically simultaneous, not only for a given plant but for all the plants of the locality."

This work by WILLIAMS was done at Bangor, Wales, and at Plymouth, England. During the summer of 1906, observations were made on the same plant at Beaufort, North Carolina, by W. D. HOYT (**4**): "It was found at Beaufort, also, that this alga produced its sexual cells in regular, periodic crops. . . . . One observation is interesting in this connection. A female plant kept in the laboratory for nearly two months and then examined showed sori only slightly less advanced than those on plants growing in the harbor. . . . . This result is similar to the one observed by WILLIAMS but goes a step farther in showing that periodicity is transmitted to new structures formed from the original plant, even where these are not subjected to alternating conditions. . . . . It still remains to be seen whether the periodic habit can be transmitted through the protoplasm of the egg."

Spirogyra under artificial environment

In the laboratories in which these experiments were conducted, windows were used which faced toward the northwest, northeast, and southeast. Whenever a new line of investigation was begun, three sets of aquaria of different sizes were prepared. One set was placed in a northwest, one in a northeast, and one in a southeast window. The conditions made use of in the three sets of experiments were the same with the exception of a few degrees in temperature and a difference in light intensity. Other experiments were conducted which had nothing to do with conditions of temperature and light.

In the first experiments Spirogyra was brought into the laboratory and small amounts placed in aquaria filled with water from the pond from which the plant was taken. In some cases the plant was washed before being transferred to the aquaria, in others it was not.

A second group of experiments contained the same conditions with the exception of the culture medium. In this group attempts were made to repeat the experiments while using tap instead of pond water. As a result, it was found that the aquaria with tap-water were as satisfactory, and, in most cases, much more so than those with pond water. Hence tap-water aquaria were used as controls.

It is well to note concerning this point that the tap-water was from reservoirs which were in turn supplied by small brooks and surface drainage. In many cases the pond water was taken from ponds supplied by brooks which drained the reservoirs just mentioned.

I next tried a group of experiments for the purpose of fixing some value to the use of plant food solutions for the investigation at hand. Knop's solution was most frequently used; however, Sachs's solution was employed in a great many experiments and in most cases simultaneously with Knop's. In comparing aquaria with and without food solutions, no general rule could be established. On the whole, however, the writer is inclined to favor at least a weak food solution. Knop's solution as low as 0.04 per cent. gave favorable results.

A long list of experiments was prepared in which rain and distilled water were used; and in others melted snow. Some of these were not disturbed after being placed in their respective windows. Others were left in these media for periods varying from one to ten days and then changed to a weak food solution (usually 0.05 per cent.), and this was raised by degrees to 0.2 per cent. where it was allowed to remain. In this group care was taken to select Spirogyra from different localities. Specimens were obtained from swift water, from slow-running brooks, and from small quiet pools and ponds where very little disturbance was possible. Notes on these experiments show that no particular advantage was gained by using these media.

From the first the aquaria located in the southeast windows were shorter lived than either of the other sets of aquaria. It will be remembered that the only difference in conditions was a slight difference in temperature plus a difference in light intensity. Experiments were now conducted in order to try the effect of shading the cultures. Tissue paper of different colors was used for this purpose. The best results were obtained when the aquaria were not shaded above the surface of the water. After scores of experiments the best results were obtained in the following way: when an aquarium was started, it was shaded with black paper; after two weeks, this was changed to yellow; and after another two weeks the shading was not used at all. This was a decided advantage to the aquaria in the southeast windows. While the effect was not so noticeable in the other laboratories, the results were sufficient to justify this method in all the windows. It is not to be understood that tap-water aquaria were the only ones used with a shade. On the other hand, snow, rain, distilled water, and food solutions were used in every case. The same applies to the following series of experiments.

Up to this time no attention has been given to the substratum, nor to other plants with which Spirogyra is most frequently associated in its natural habitat. For this group of aquaria soil, débris, and plants, for the most part, were used from the immediate locality from which the specimens were taken. In some cases the soil was first sterilized.

Among the plants which seemed beneficial to Spirogyra, none proved more helpful than Oedogonium. In fact in every aquaria in which Spirogyra lived for several months or a year, Oedogonium was invariably present and often the predominating alga. The Oedogonium could usually be found attached to the sides of the jar or floating at the surface, thus shielding the Spirogyra from too much sunlight. Chara supplied to the aquaria was helpful so long as it was not too abundant. In some cases where aquaria were thus started, the Chara died out after two or three months and was followed by an abundant growth of Oedogonium, and later by Spirogyra. In the field, masses of Spirogyra were often collected among water cress, but in no case was a good growth of Spirogyra obtained in an aquarium with this plant. A number of jars were supplied with oak leaves taken directly from the trees where they had remained all winter. To other aquaria pieces of charcoal were added. Both of these factors seemed beneficial and in most cases excellent cultures of Spirogyra were obtained which lasted until conjugation had taken place.

Spirogyra under natural environment

Field-work was begun early in April. At this time 400 aquaria of all sizes were in use and 100 of them contained excellent cultures of Spirogyra which had been under cultivation for two months. On

April 27 *S. quadrata* was found conjugating abundantly. This one was followed by others until twelve different species had matured and disappeared. At first all material was brought to the laboratory in small jars and then examined, but this method was by no means satisfactory. A microscope together with a crude temporary stand was added to my collecting outfit, and an examination of all material was made on the spot. There are so many chances of collecting worthless material that no other method was found which would give definite results.

The first task was to locate Spirogyra in a number of different localities with different conditions of environment. Accordingly a few localities were selected varying from a few rods to twelve miles apart. Within these regions 40 different places were selected which could be visited frequently and studied with care. Some were in open places, while others were in deep shadow; some were in pure running water, others in filthy stagnant pools; some with numerous other algae, others almost alone; and under many other conditions.

For identification of species some of the material was usually killed in the field as soon as collected. From 2 to 4 per cent. solution of formalin was used for this purpose when larger masses were killed, but in most cases chromacetic solution was used according to the formula given in CHAMBERLAIN'S *Methods in plant histology*, p. 139 (chromic acid 1^{gr}, glacial acetic acid 4^{cc}, water 400^{cc}). Several stains were used, but Haidenhain's iron-hematoxylin was most satisfactory. Some of the specimens stained in this way were afterward mounted in glycerin, others in balsam, and others in Venetian turpentine. I found the balsam more satisfactory than the turpentine, but excellent preparations were obtained by using the Venetian turpentine method recommended by CHAMBERLAIN (stain with magdala red and anilin blue and mount in Venetian turpentine). Stained preparations in glycerin were most quickly obtained by taking the material as soon as washed from the chromacetic solution, transferring it to a 10 per cent. solution of glycerin to which was added 2 or 3 drops of Ehrlich's blood stain, and allowing it to concentrate. In some cases the stain obtained by this method has been permanent, in others it has faded after a few months. This method of preparation has been used with much success by F. N. DUNCAN and O. P. DELLINGER, of this laboratory, for animal tissue.

Periodicity of species studied

For the purpose of identifying the different species, the following authors were consulted: VAUCHER (**6**), WOOD (**9**), PETIT (**5**), and WOLLE (**10**). A brief account of the fruiting season of the different species will be given below.

Spirogyra inflata (Vaucher) Rab.—VAUCHER reports this species in fruit from February 20 to March 19. PETIT gives April–May. Unfortunately I was not able to find it except in a single locality. This was in a small pond covering an area of perhaps 1000$^{\mathrm{sq.\,ft}}$. The surface was well covered with algae and floating débris of small sticks and grass. This species was found floating on the water together with *S. catenaeformis* and *S. calospora.* On May 2 all three species were found in a state of conjugation interwined with each other. No indication of *S. inflata* was found after May 12, although this pond was visited frequently during the summer and fall.

Spirogyra quadrata (Hassall) P. Petit.—This is the *Zygnema quadratum* of HASSALL. PETIT gives the fruiting season as April–May. The writer studied this species in eight different localities, varying from a few rods to twelve miles apart, but under no great variety of conditions. In every case it was found in quiet surface-water pools or streams which were entirely dry by the middle of July. All the eight places were in low pastures or meadows and specimens were collected from among dead grass and leaves. *S. quadrata* was found fruiting abundantly April 27, and within a week the eight places mentioned above were visited and found in about the same state of conjugation. By May 5 the Spirogyra at two localities had fruited abundantly and had passed the maximum of conjugation. By May 12 this was true in every case and no fruiting material was found after May 19. The temperature of the water recorded at each visit to the different ponds gave a range of 10–25° C. It was very difficult to collect any vegetative filaments of this species after the period of maximum conjugation had passed.

Spirogyra calospora (Cleve).—WOOD found this species fruiting in a ditch in a meadow late in April, and as late as May 25 in the "neck" below Philadelphia. The season given by PETIT is April–May, and the habitat "ditches in low ground and swamps." For this species the present paper includes notes from observations of five different ponds and one brook in different localities, and including a greater variety of conditions than those described for *S. quadrata*. Early in April the plant in question was growing abundantly and continued in increasing abundance until about the second week in May. In two of the ponds the disappearance was by no means rapid. A few scattering filaments were found May 6 and the last June 4. The average temperature was 7–14° C. In the pond in which most fruiting material was found the temperature ranged from 7 to 11° C at the time of maximum conjugation.

Spirogyra Hantzschii Rab.—In 1906 fruiting material was collected first on April 28, and, with one exception, no fruiting material was gathered after May 23. In the fall of 1905 this species was found in active vegetative condition in three localities. Examinations were made frequently during the fall and, when the ponds were free from ice, during the winter. This species lived beneath the ice and in two of the three localities the plant did not come to the surface at the time of conjugation, but was collected from the débris at the bottom of the ponds. Temperature of the water varied from 12–28° C. There was no great range of habitat; generally in shallow ponds near the mouth of a small stream, and more or less protected from the midday sun.

Spirogyra mirabilis (Hassall).—This species is described by HASSALL and also by PETIT as producing spores without conjugation and by no other method. WOLLE states that lateral conjugation is more frequent than scalariform. I was not able to find this species in fruit in the pond from which this material was taken. It was brought into the laboratory in the vegetative condition April 2 and within a week was fruiting in several aquaria. On May 7 a few filaments with scalariform conjugation were found; all other spores were formed in cells where no conjugation had taken place. Comparatively few cells formed spores and very few cells conjugated. PETIT gives March–July as the fruiting season. In the material mentioned all fruiting specimens had disappeared in all the aquaria by the second week in June.

Spirogyra Jurgensii (Ktz.).—This species was found in fruit in the neck of a small pond May 4, 1906. In the same locality were found vegetative filaments of *S. Hantzschii* and Oedogonium, besides sticks, dead grass, rusted wire, and tin cans. The algae mentioned were found here in small patches January 8, and were visited at least once each week until June, at which time no algae of any kind were present. The fruiting specimens were most abundant when the temperature averaged about 12° C., and lasted about a week. No fruiting specimens were found after May 25. PETIT gives the conjugating season as April–May.

Spirogyra catenaeformis (Hassall) Ktz.—This species, so far as I know, has not been reported for this country, and is so placed because it corresponds so closely to the descriptions in the works of HASSALL and PETIT. The latter author gives the fruiting season as April–May, and the habitat "very humid ditches, wooded ponds, etc." The first fruiting material was collected May 4 and the last May 22. It was found in two localities about two miles apart. One was a shallow ditch and the other was in cow tracks near a small stream. The period of conjugation was the same for both localities. The temperature of the water ranged from 14 to 17° C. This species was found with *S. dubia* in one pond and with *S. inflata* and *S. calospora* in another pond.

Spirogyra varians (Hassall).—This plant was studied in five localities from one to eight miles apart. In one case the water was rather swift and the Spirogyra was fruiting wherever it happened to lodge against a support. The other localities were shallow ditches and ponds. The first fruiting material was collected May 2, and the last May 23. In all localities except that in swift water this species fruited abundantly. The average temperature of the water was 8–12° C.

Spirogyra longata (Vaucher).—For this species my observations were confined to two ponds about one mile apart. In one *S. longata* covered an area of perhaps $200^{\text{sq ft}}$ and in the other fully 100 sq. rods of surface. In both ponds the plant in question was well exposed to bright sunlight, receiving its main protection from numerous algae which were present. The temperature of the water ranged from 20 to 25° C. and the depth of the water from a few inches to several feet. Vaucher says that this species conjugates from February 20 to April 12; Petit gives April–May. In the ponds mentioned this species appeared in July and with increasing abundance until early in August. The first fruiting specimens were collected August 2, and the last August 21, and neither vegetative nor conjugating filaments as late as September 1.

Spirogyra Lutetiana (P. Petit).—I was not able to identify this species except in a single locality. Its habitat was a small open ditch in a meadow unprotected from the sun. Petit gives the fruiting season as March–May. The only date at which I found it in a state of conjugation was October 14, and when collected it had all the appearance of being old material which had fruited earlier. This may have been a week and it may have been a month.

Spirogyra dubia Ktz.—Concerning this species Wood writes: "I have found this species fruiting abundantly in May. When in this condition it forms a mass of dirty yellowish-green." Wolle agrees as follows: "Fruiting abundantly in May; forming dirty yellowish-green masses." I have found this species in fruit in seven different localities. The first date for each was as follows: May 1, May 2, May 21, July 31, July 31, August 10, and August 10. Those fruiting in May were found in rather sluggish streams and in water varying in depth from one to four feet; those fruiting in July and August were in cattle tracks and in a small ditch. The conjugating period in the spring lasted about four weeks; in the summer the period was about two weeks. The temperature of the water in the former ponds varied from 12 to 18° C.; in the latter places from 22 to 28° C. All seven localities were situated where the plant was protected from direct sunlight for a part of the day.

Spirogyra orthospira (Nägeli).—I had an excellent opportunity to watch the life-history of this species in a small pond of about 100$^{\text{sq ft}}$ in area, two to four feet deep, and well shaded with low alders. The surface of the pond was entirely covered with Spirogyra to a depth of several inches. On May 22 it was found to be fruiting abundantly both at and beneath the surface. It reached its maximum about the second week of June, and by June 30 either vegetative or conjugating filaments could be found only by examining the débris at the bottom of the pond.

General conclusions

In forming conclusions concerning whether any given plant passes through a series of regular recurring phenomena, it is well to withhold positive conclusions until the plant in question has been carefully observed for a number of successive seasons. This method of approach becomes more complicated, but at the same time safer, if the plant can be studied from a number of varying conditions of environment. However valuable external conditions may be in effecting any given phenomenon, it seems well not to overlook whatever influence may be due to internal conditions. There may be conditions that cannot be seen, felt, weighed, or measured, that initiate and support even the simplest plants through a series of complicated phenomena. Notes on Spirogyra for this region seem to credit much influence to internal conditions. Here were forty different places in which this plant appeared, matured, and disappeared, in a single season. Without a single exception the disappearance was immediately preceded by a period of conjugation. Special importance is given to the fact that in no case did all of the filaments enter into spore formation either with or without conjugation. It is to be further noted that both the vegetative and conjugating filaments dissociated almost at the same time and disappeared, even from places where this plant was abundant over comparatively large areas of surface.

Of the twelve species studied, ten were in fruit in May, and in every case the maximum abundance of conjugation occurred within this month. In a few places more than one species was in fruit at the same place and time, but usually if several forms grew together they conjugated at different times, their period of maximum conjugation succeeding one another at intervals ranging from one to two weeks. For example, early in March, within a distance of forty rods, four species of Spirogyra were found growing together at three different places at the edge of a slow brook. In two of the three places Spirogyra was very abundant and covered an area in each of about 200$^{\text{sq ft}}$ Probably all had had the same source earlier in the year during high water, but remained distinct until all had disappeared in June. No fruiting material was found in either of the larger patches; two of the four fruited in the smaller one, but not at the same time.

S. Jurgensii was found in fruit May 1–May 15; *S. dubia* was found fruiting May 21–June 4.

Under the description of *S. inflata* mention was made of the fact that on May 2 this species together with *S. calospora* and *S. catenaeformis* were found in fruit at the same time and place. It ought to be noted, however, that *S. inflata* had passed its period of maximum conjugation and that there were comparatively few filaments of either of the other two species. No *S. inflata* was found after May 4, but from this until May 22, *S. catenaeformis* was abundant. In giving a description of the latter species it was noted that this form was found fruiting abundantly at the same time and quite near *S. varians*. I did not find the two species intermingling, although growing abundantly almost in the same mass and having the same fruiting season.

I am inclined to think that not enough emphasis is given to the fact that the lower plant forms do not have a continuous period of growth, either vegetative or reproductive. In other words, the normal period for either activity is continuous for only a few weeks or months at most; it remains a problem of research to show how inclusively this generalization applies to the freshwater algae. For Spirogyra the observations reported in this paper seem to offer overwhelming evidence in support of the view, for this region at least, that it is not a perennial plant. I have only a single exception to this conclusion and will speak of it later. A few of the species studied appeared more or less abundantly in the fall; in this connection *S. Hantzschii* furnishes a specific example. In a majority of the forty localities kept under observation during 1905–6, several forms of algae succeeded one another in maximum abundance in the course of a year. In only four of the forty places was any trace of Spirogyra found in the fall where it had been in a state of conjugation in the spring. The manner in which different species appeared, matured, and conjugated was for most of the forms quite distinct and constant for the several localities. Following the fruiting season the disappearance of the plant took place sometimes suddenly and sometimes gradually.

It is a well-known fact that vegetative material is very difficult to identify; the same might as well be said of conjugating material. For this reason it is difficult to follow any given species with certainty;

where several closely allied species are associated, this is practically impossible. Fortunately this did not occur except in a few places. However uncertain and unreliable may be one's observations and conclusions relative to a given species during its period of vegetative growth, it is possible to determine with some certainty whether or not the different species of Spirogyra have any definite or fixed periods at which they conjugate, or whether this phenomenon takes place only under certain conditions of environment. In all cases notes were taken at each visit to a pond, and the determination of the species left until the fruiting season; then by going back to my notebook, the life-history could be worked out with some certainty. Leaving out of account uncertain conditions such as the vegetative activities, and placing special importance on the period at which conjugation takes place, it is possible to conclude that one of the two important phenomena (vegetative and reproductive activities) in the life-history of this alga appears periodically, not due exclusively to seasonal conditions nor to environment.

No one can say with absolute certainty whether the natural forces that produce conjugation are internal or external. It is a fact, however, that whenever a condition was present, in any part of a mass, which was able to initiate and control reproductive activity, there was at the same time and place either the same or some other condition which brought about the destruction of all vegetative filaments. In this study no exception was found to this rule, whether under laboratory or under natural environment.

Up to the time when conjugation was abundant in ponds, brooks, and ditches, I still had over 300 aquaria in the laboratory. A small percentage of them contained fruiting Spirogyra. The time of fruiting for any given species began about one week earlier and lasted about one week longer in the laboratory than that of the field. The disappearance of the vegetative filaments was also more gradual. It was not difficult to keep aquaria early in the year, but after the middle of May they began to decline and were kept only with increasing care until mid-summer, when out of 700 only one remained.

As to the different sizes of aquaria, it ought to be said that some were ordinary test-tubes; some contained 25–30 liters; and the majority ranged from one to ten liters. The results were not constant for either the large or small sizes, although all sizes were usually arranged at the same time. In dozens of test-tubes two-thirds full of water or culture media Spirogyra kept well two to three months. On the average, however, aquaria of four to ten liters were most satisfactory. In three large aquaria of about forty liters each no successful cultures were made, although frequently attempted.

An attempt was repeatedly made to grow Spirogyra in some of the larger aquaria after a species had died in it and without renewing the culture media. In every instance where this was tried the plant died down in a few days, showing that the solution contained a toxin injurious to Spirogyra. It is left as an open question whether a mass of Spirogyra in nature dies from the accumulation of this same destructive element.

Aside from the twelve species herein described, there was still another which has been kept under observation since early in the fall of 1905. It is a large form and was found growing in rather large bright-green patches in the running water of three different brooks one to three miles apart. When first found, a few aquaria were started, one of which has remained to the present time without any indication of decline. This is an ordinary aquarium of about 10 liters; it is kept well covered and for that reason very little evaporation takes place. The main algal associate is Oedogonium, and a few brown and green hydra have lived in the aquarium from the beginning. This jar is the one exception to the 700 aquaria that died down during the season of 1906. In concluding, I wish to call attention to the fact that no trace of a conjugating filament has been found in this aquarium; and furthermore, that none was found at any of the three brooks mentioned, although in one case the plant was very abundant. It is also remarkable that there has not been a time from the first when vegetative specimens of this species could not be obtained.

In addition it ought to be said that this study was continued during the following year (1906–7), in the same locality, under about the same conditions and methods of study. This second year's observations and experiments did not vary enough from those of the previous year to warrant any change in the conclusions above given.

Summary

1. Few of those who have published papers on the genus Spirogyra have given any notes on the subject of periodicity.

2. Out of 700 aquaria it was found that the best way to arrange a culture was to place some sterilized earth in the bottom, add some dead leaves or dead grass, allow to settle well, then add a small amount of Spirogyra, and place at first in a window not exposed to direct sunlight.

3. Of 300 aquaria in the laboratory at the time when Spirogyra was fruiting most abundantly under natural conditions, there were about 5 per cent. which contained conjugating material. The time corresponded with that of the field.

4. Leaving out of account the number of species which may have been collected in the vegetating condition and for that reason doubtful of identification, there were at least thirteen different species collected, of which twelve fruited more or less abundantly.

5. Ten of the thirteen species passed their period of maximum abundance in May, one in August, and one in October. A few doubtful fruiting filaments of *S. Hantzschii* were found a second time in August. The only reliable example of a second fruiting period found was *S. dubia*, which fruited in May, and again in small patches, but relatively abundant, during the latter part of July.

6. When field-work was first begun, material was collected and then brought to the laboratory for examination. This method was by no means satisfactory and for that reason was discarded. All the later collections were examined on the spot with a microscope.

7. The period of maximum abundance corresponds in every case with the period of maximum conjugation. The decline of one was always accompanied by that of the other.

8. After conjugation had ceased the fruiting filaments disappeared more or less gradually. The vegetative filaments also disappeared, and at the same time as those containing zygospores.

9. In the laboratory no Spirogyra was found fruiting at the surface but always near the substratum. In the field the reverse was true. *S. orthospira* and *S. Hantzschii*, however, were prominent exceptions.

10. Of the thirteen species one has not been found in a state of conjugation. It has been kept under observation, in excellent con-

dition, both in the laboratory and in the field, since October, 1905, but no trace of fruiting material has been found.

11. Notes taken in the field and supplemented by those in the laboratory offer overwhelming evidence in support of the view that the phenomenon of conjugation results not so much from external as from internal conditions.

12. The writer therefore concludes that Spirogyra has definite periods of growth and activity.

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